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Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits

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Abstract

Febrile (fever-induced) seizures affect 3–5% of infants and young children. Despite the high incidence of febrile seizures, their contribution to the development of epilepsy later in life has remained controversial. Combining a new rat model of complex febrile seizures and patch clamp techniques, we determined that hyperthermia-induced seizures in the immature rat cause a selective presynaptic increase in inhibitory synaptic transmission in the hippocampus that lasts into adulthood. The long-lasting nature of these potent alterations in synaptic communication after febrile seizures does not support the prevalent view of the 'benign' nature of early-life febrile convulsions.

Fever-induced seizures are the most common form of childhood seizures[1–3]. Because of the large number of affected children, whether febrile seizures cause long-term alterations in neuronal excitability is of paramount importance. However, the association of febrile seizures with temporal lobe epilepsy remains undetermined. Although retrospective studies have repeatedly shown that many patients undergoing surgery for intractable temporal lobe epilepsy have mesial temporal sclerosis and a history of prolonged, febrile seizures[4–6], population-based and prospective studies typically have failed to find associations between early-life febrile seizures and temporal lobe epilepsy[7,8]. Thus, the prevailing opinion suggests that febrile seizures are benign, and they are generally not treated aggressively[9]. However, imaging studies have found evidence of rapid alterations in hippocampal structures after prolonged focal febrile convulsions[10]. Indeed, it is these prolonged (longer than 15 minutes), focal (complex) febrile seizures that are most consistently associated with subsequent temporal lobe epilepsy[4–6].

To address these seemingly irreconcilable observations from human studies, we undertook systematic prospective studies using a new model system in the rat (the hyperthermia model of febrile seizures[11,12]). Specifically, we sought to determine whether hyperthermia-induced seizures lead to long-term alterations in limbic neuronal excitability. The advantage of this approach is that, unlike in the clinical situation, the presence of pre-existing lesions can be excluded, and the duration and magnitude of both the hyperthermia and hyperthermic seizures can be highly regulated. In this model, 10-day-old rats were exposed to a regulated stream of mildly heated air (42–43 °C). As core temperature reached 40–41.5 °C (that is, temperature-ranges comparable to those measured in sick children with high fever), stereotyped, mainly tonic seizures developed reproducibly in more than 98% of the rats. These behavioral seizures were associated with electro-graphic epileptic discharges from the

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hippocampus[11]. In addition, the duration of these behavioral and electroencephalographic seizures could be regulated.

The data demonstrate that highly specific, long-term modifications in neuronal excitability occur in the developing limbic system after exposure of the developing brain to hyperthermia-induced seizures. These results may fundamentally alter the current perspective regarding the outcome of febrile seizures.

Lasting decrease in pyramidal cell excitability

What effect, if any, do febrile seizures in this model have on neuronal excitability in the limbic system? We obtained field recordings of population responses after stimulation of the Schaffer collaterals from the CA1 region of hippocampal slices from control rats on postnatal day 17 and from their littermates that experienced hyperthermia-induced seizures (HT group) a week earlier. In control artificial cerebrospinal fluid (ACSF), the population spikes from HT rats were smaller in amplitude than those of control rats, at all stimulation intensities (Fig. 1). The depressed CA1 population firing indicates that hyperthermia-induced seizures cause long-lasting (that is, lasting at least 1 week) alterations in hippocampal excitability.

The $GABA_A$ receptor blocker bicuculline (at a concentration of 20 μM) abolished the difference between the experimental and control groups (Fig. 1*a* and *c*). Specifically, when the population responses were recorded in the presence of bicuculline, the population discharges appeared similar in control and HT rats. These findings indicate that the depression of the population spikes involves an enhanced $GABA_A$ receptor-mediated inhibition of pyramidal cells.

Long-lasting enhancement of inhibitory postsynaptic currents

Whole-cell patch clamp experiments showed that the amplitude of the pharmacologically isolated, monosynaptically evoked inhibitory postsynaptic currents (IPSCs) from CA1 pyramidal cells 1 week after hyperthermia-induced seizures was significantly increased at all stimulation intensities, compared with IPSCs from control rat (Fig. 2). Furthermore, the potentiation of the IPSCs was long-lasting, as the increase in IPSC amplitude could be found even 10 weeks after hyperthermia-induced seizures (Fig. 2*c*); that is, in fully mature rats, without any decrease in the potentiation at 10 weeks relative to earlier times after seizure (Fig. 2*c*, inset). These data are consistent with an enhanced $GABA_A$ receptor-mediated inhibitory control of pyramidal cell discharges, as predicted from the field recording experiments. The potentiation of the IPSCs was due to the seizures, and not to the hyperthermia itself, as the IPSC amplitude from hyperthermic control rats (rats exposed to hyperthermia, but in whom the seizures were blocked with pentobarbital) was identical to that of normothermic control rats (Fig. 2). Furthermore, pentobarbital alone failed to alter the amplitude of the evoked IPSCs in normothermic control rats (Fig. 2*b*, inset). These field recordings and patch clamp data indicate that the inhibitory system undergoes activity-dependent, long-term enhancement after hyperthermia-induced seizures in immature rats.

Presynaptic locus of the IPSC potentiation

To determine whether the primary locus of the long-term enhancement of the IPSCs is pre- or postsynaptic, we recorded miniature IPSCs (mIPSCs) from CA1 pyramidal cells. The frequency of the mIPSCs was nearly doubled (that is, the inter-event interval was nearly halved; Fig. 3*b*) in cells from rats that experienced seizures, compared with that of control rats (Fig 3). The increased mIPSC frequency gave further support to the previous results indicating the long-term enhancement of the inhibitory synaptic transmission after

hyperthermia-induced seizures. There was no significant increase in mIPSC frequency in the hyperthermic control rats (Fig. 3*b*).

In contrast to the increase in the mIPSC frequency, the mIPSCs of control and experimental rats were identical in amplitude (Fig. 3*c*) and kinetics (not shown: 10–90% rise-time in control, 0.82 ± 0.11 ms; in HT, 0.78 ± 0.09 ms; decay time constant in control, 6.3 ± 0.3 ms; in HT, 5.9 ± 0.4 ms). The profound enhancement of the mIPSC frequency, without a change in the mIPSC amplitude or kinetics, indicates that the locus of the long-term plasticity underlying the observed increase in evoked IPSCs is presynaptic.

To determine whether the hyperthermic seizure-induced alterations in inhibition were specific to the hyperthermia-induced seizures, we induced other limbic seizures in rats of the same age using the prototypic limbic convulsant kainic acid. Kainic acid-induced seizures did not result in the enhancement of the mIPSC frequency observed after hyperthermia-induced seizures (Fig. 3*b*, inset). Furthermore, unlike the hyperthermia-induced seizures, kainic acid-induced seizures caused a small, but statistically significant increase in the amplitude of the mIPSCs (Fig. 3*c*, inset). These results indicate that hyperthermia-induced seizures may lead to unique alterations in hippocampal inhibition.

Protein kinase A dependency of the potentiated inhibition

What is the nature of the molecular pathways that underlie the presynaptic enhancement of the inhibitory system? Exogenously applied activators of protein kinase A (PKA) and protein kinase C (PKC) cause a presynaptic increase in both the amplitude of evoked IPSCs, and in the frequency (but not the amplitude or kinetics) of mIPSCs (ref. 13). As these are the precise changes found here, we did further experiments to test the hypothesis that PKA and/or PKC may be involved in the potentiation of the inhibitory responses in the HT rats.

The broad-spectrum PK blocker staurosporin at a concentration of 0.5 μM abolished the difference between the evoked IPSCs recorded from control and experimental rats (Fig. 4*a*). Similarly, staurosporin abolished the seizure-related difference between the mIPSC frequency in cells from control (5.83 ± 0.97 Hz, $n = 12$) and HT (6.45 ± 0.97 Hz, $n = 13$) rats. These staurosporin effects indicate the involvement of a protein kinase in the presynaptic potentiation of inhibitory neurotransmission.

To distinguish between the involvement of PKA and/or PKC in the enhancement of the IPSCs, we did experiments with blockers specific for either PKA or PKC. The specific PKA antagonist Rp-cAMPS (ref. 14) at a concentration of 100 μM blocked the increase in evoked IPSC amplitude in experimental rats, and decreased the IPSC amplitude to control levels (Fig. 4*b*). Staurosporin and Rp-cAMPS did not have any effect on the IPSC amplitude in control rats. In contrast, the PKC-specific antagonist calphostin-C at a concentration of 1 μM failed to decrease the enhanced evoked IPSC amplitude in experimental rats (Fig. 4*c*). Calphostin-C was able to block PKC in these conditions, as the PKC activator phorbol ester (0.5 μM phorbol 12,13-dibutyrate; PDBU) did not increase the amplitude of the evoked IPSCs in cells from brain slices incubated in calphostin-C (IPSC amplitude in the presence of PDBU, with respect to the control IPSCs before PDBU in the same cells: 92.0 ± 6%; $n = 3$), but it did so in cells not exposed to calphostin C (211.0 ± 43%, $n = 4$). Therefore, the long-term presynaptic enhancement of the IPSCs after febrile seizures is dependent on activation of PKA but not PKC.

As a further test for the PKA-dependent, presynaptic nature of the long-term enhancement of the IPSCs, we used forskolin, which enhances inhibitory synaptic transmission in the hippocampus presynaptically[13] through the adenylyl cyclase–PKA system. Forskolin at a concentration of 10 μM caused a larger increase in the evoked IPSC amplitude in

experimental rats than in control rats (Fig. 4*d*), providing additional evidence that there is a PKA-dependent presynaptic alteration underlying the enhancement of IPSCs in HT rats. To further investigate the mechanism of the PKA-dependent increase in IPSCs, we determined whether the enhancement persisted even in the presence of the calcium channel-blocker cadmium. Acute stimulation of the PKA-system in hippocampal slices has been shown to lead to enhancement of the mIPSC frequency even in the presence of cadmium[13], indicating that the mechanism of action is unlikely to involve a depolarization of the interneuronal terminals (for example, due to a reduction of a presynaptic potassium conductance)(ref. 15). The enhanced mIPSC frequency in HT rats was also insensitive to the blockade of voltage-gated calcium channels, and could not be decreased by 100 μM cadmium (mIPSC frequency in the presence of cadmium, with respect to the control level before cadmium, 123.0 ± 15.1%; $n = 5$). Furthermore, a PKA-dependent activation of β-adrenergic receptors16 is unlikely to underlie the PKA-dependent potentiation of inhibition after hyperthermia-induced seizures, as incubation (for more than 1.5 hours) of brain slices in the presence of 1 μM of the β-receptor antagonist propranolol failed to decrease the enhanced IPSCs from HT rats. The amplitude of evoked IPSCs in the presence of propranolol ($n = 6$) with respect to the cells in brain slices from the same HT rats not exposed to the drug ($n = 5$) was 105.7 ± 7.5%; the IPSC amplitude in these HT rats was verified to be enhanced with respect to control values (for example, 170.2 ± 3.7% at 2 mA stimulation intensity).

Selective enhancement of the inhibitory system

How selective are the hyperthermic seizure-induced changes in synaptic transmission to the inhibitory system? In contrast to the evoked IPSCs, the amplitude of the evoked excitatory post-synaptic currents (EPSCs) in CA1 pyramidal cells 1 week after hyperthermia-induced seizures was identical in control and experimental rats at all stimulation intensities (data not shown; control, $n = 10$; in HT, $n = 10$). In addition, we did not find a substantial decrease in cells from experimental (HT) rats compared with cells from control rats for the miniature EPSC frequency (control, 1.6 ± 0.4 Hz, $n = 12$; HT, 1.9 ± 0.4 Hz, $n = 12$) or amplitude (control, 12.8 ± 0.4 pA, $n = 12$; HT, 14.1 ± 1.3 pA, $n = 12$), the input resistance (control, 89 ± 12 MΩ, $n = 13$; HT, 98 ± 11 MΩ, $n = 16$), the resting membrane potential (control, −64.5 ± 2.2 mV, $n = 16$; HT, −62.7 ± 2.4 mV, $n = 19$) or the action potential threshold (control, −47.9 ± 2.9mV, $n = 9$; HT, −49.2 ± 2.4 mV, $n = 7$). These data indicate a high degree of selectivity of the hyperthermic seizure-induced long-term alterations of neuronal excitability for the hippocampal inhibitory system.

Discussion

The hyperthermia model of febrile seizures results in highly reproducible and controlled seizures in the setting of a normal, developing brain at a relevant temperature threshold. Induction of these seizures in almost all rats indicates that predisposing abnormalities are not required for their generation. In addition, the model approximates the human situation of age dependence of febrile seizures, which are confined to infancy and early childhood: in the model, the hyperthermic seizures are best elicited on postnatal days 10–14 (refs. 11,17). The comparison of developmental ages of human and rat is complex and approximate. However, in both humans and the experimental model, an age dependence of febrile seizures exists, similar to models of hypoxia-induced seizures[18,19], which is consistent with the idea that certain characteristics of the developing circuit may facilitate the generation of these seizures. In addition, the temperature threshold for seizures in this model is comparable to the temperatures resulting in febrile seizures in children. Obviously, hyperthermia does not encompass the spectrum of biological processes related to fever. However, although the mechanism by which fever and the model lead to elevated brain temperature may differ, they both lead to hyperthermia-induced seizures. This is especially noteworthy, as the

alterations in limbic excitability reported here were clearly the result of the seizures that were induced by hyperthermia (possibly involving a synergistic effect of hyperthermia and seizures), and not of the hyperthermia itself, because hyperthermic controls in which seizures were blocked did not show any structural[12] or electrophysiological changes when compared with normothermic controls. Thus, fever and experimental induction of elevated brain temperature both cause hyperthermia-induced seizures, and it is the seizures, potentially augmented by hyperthermia, that cause long-term alterations in neuronal excitability.

Histochemically detected changes in principal cells after hyperthermia-induced seizures are transient[12], in contrast to the persistent functional alterations at the interneuronal terminals. Although the connection, if any, between the transient physicochemical alterations in principal cells[12] and the long-lasting changes in GABAergic neurotransmission is not clear, the high specificity of the synaptic alterations reported here indicates that functionally, hyperthermia-induced seizures preferentially modify inhibitory circuits within the limbic system[20–25]. Our results also show that kainic acid-induced seizures at the same developmental age did not enhance mIPSC frequency, indicating that intense activation of the limpic circuit itseld is not sufficient to lead to the long-lasting, presynaptic alterations in inhibition seen after hyperthermia-induced seizures. In agreement with the kainic acid data, no similar seizure-induced presynaptic alterations in the inhibitory system have been reported after experimentally induced seizures in adult rats[26–29] or after hypoxia-induced seizures in infant rats[18,19], indicating that the hyperthermia-induced seizures in infancy may lead to unique changes in the limbic system.

The long-term enhancement of the efficacy of hippocampal inhibition may be viewed as a compensatory response to seizures on the part of the developing neuronal network. However, IPSCs can lead to synchronization of postsynaptic cells[30], which may be considered a proconvulsant action. Indeed, increased hippocampal inhibition has been reported in other models of hyperexcitability as well; for example, after kindling in adult animals[26,27,29]. In general agreement with these data indicating simultaneous increases in inhibition and excitability, hyperthermia-induced seizures may also lead to an ultimately hyperexcitable neuronal network, especially when the system is strongly challenged by either tetanic electrical stimulation *in vitro* in combined entorhino–hippocampal slices or by kainic acid *in vivo* (K.C., T.Z.B. and I.S., unpublished observations). The precise understanding of the relationship of potentiated inhibition and lower seizure threshold provides an exciting, important challenge for future research. The high specificity of the febrile seizure-induced alterations to the principal inhibitory fast neurotransmitter system holds promise that new therapeutical avenues can be explored that selectively modulate seizures but do not alter limbic cognitive functions.

Synaptic plasticity is important in learning and memory, in the development of transmitter-gated communication between cells, and in pathological states of neuronal excitability[31,32]. Most studies aimed at understanding the mechanisms underlying long-term changes in synaptic efficacy focused on glutamatergic synapses[31], and far fewer data are available on the long-term alterations at GABAergic synapses. A long-lasting increase in the number of the postsynaptic $GABA_A$ receptors underlies the potentiated mIPSC amplitude in kindled rats[26,27,29]. Similarly, the subunit composition of the postsynaptic $GABA_A$ receptors can also be altered in a long-term manner at the inhibitory synapse[28,29]. Given the high probability of opening and high occupancy of synaptic $GABA_A$ receptors[33–36], postsynaptic alterations in receptor number and subunit composition are effective ways of modifying transmission at inhibitory synapses.

Our data demonstrate that the presynaptic hippocampal interneuronal terminals can also be a site of long-lasting modification. The PKA-dependent, presynaptic nature of the potentiation of inhibition after hyperthermia-induced seizures resemble the PKA-dependent, presynaptic long-term potentiation of mossy fibers[37,38]. Presynaptic enhancement of GABAergic inhibition reported to occur during withdrawal from morphine in the ventral tegmental area also involves a cAMP/PKA-dependent process[14]. The finding that the presynaptic GABAergic terminal can undergo activity-dependent alterations is likely to alter ideas about how the synaptic gain can be controlled at GABAergic synapses in neuronal circuits[26–29,39–43]. The exact mechanisms underlying the cAMP/PKA-dependent presynaptic enhancement of GABAergic transmission after hyperthermia-induced seizures are not yet fully understood. Our data indicate that depolarization of interneuronal terminals (for example, because of a kinase-related reduction in a presynaptic potassium channel)[15], or a PKA-dependent persistent activation of β-adrenergic receptors16, are unlikely to be responsible for the increase in IPSCs after hyperthermia-induced seizures. The relevant targets of PKA may include proteins involved in the synaptic release machinery, and phosphorylation of the cAMP response element binding protein by PKA may also be central in hippocampal plasticity[44].

The PKA-dependent, presynaptic, long-lasting enhancement of hippocampal inhibitory transmission is especially relevant, given reports indicating that the excitatory input to hippocampal interneurons may not show direct forms of long-term potentiation or long-term depression[45,46], which may be related to a lack of expression in interneurons of second messenger molecules such as the alpha subunit of the calcium–calmodulin-dependent protein kinase II (ref. 47) and calcineurin[48]. The presynaptic enhancement of inhibition resulted in a bicuculline-sensitive, long-term depression of the CA1 population spikes, demonstrating that the PKA-dependent presynaptic plasticity at GABAergic synapses in the hippocampus can alter how pyramidal cells respond to incoming signals from the CA3 region.

In conclusion, the results presented here demonstrate a presynaptic form of long-term enhancement of inhibitory synaptic transmission, and support the idea that early-life febrile seizures lead to persistent effects on neuronal excitability in the limbic system.

Methods

Slice preparation

Brain slices were prepared as described[49]. Control and experimental littermate Sprague-Dawley rats (Zivic-Miller, Zelienople, Pennsylvania) were anesthetized with halothane and decapitated, and their brains were removed and cooled at 4 °C in oxygenated (95% O_2/5% CO_2) artificial cerebral spinal fluid (ACSF): 126 mM NaCl, 2.5 mM KCl, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1.25 mM NaH_2PO_4 and 10 mM glucose. Horizontal brain slices 450 μM in thickness were prepared with a Vibratome tissue sectioner (Lancer Series 1000; Polysciences, Warrington, Pennsylvania). The brains were sagittally bisected into two hemispheric components and preincubated submerged in oxygenated ACSF at 32 °C for at least 1 h in a holding chamber before any experimental manipulations.

Electrophysiology

Individual brain slices were transferred to a recording chamber[49] perfused with ACSF containing the appropriate drugs for each experiment. The brain slices rested on filter paper and were stabilized with platinum wire weights. The tissue was continuously superfused with humidified 95% O_2/5% CO_2, and the temperature of the perfusion solution was maintained at 35 °C. Whole-cell recordings were obtained by experimenters ‘blinded’ to

sample identity as described[49]. Recordings were obtained with an Axopatch-200A amplifier (Axon Instruments, Foster City, California), or with a Neurodata intracellular amplifier (Neuro Data, Delaware Water Gap, Pennsylvania), and digitized at 88 kHz (Neurocorder; Neuro Data, Delaware Water Gap, Pennsylvania) before being stored in pulse-code modulated form on videotape. The series resistance was monitored throughout the recordings, and the data were rejected if there was a substantial increase in series resistance during the recording. Individual experiments each day were on brain slices from control and HT rats in an alternating sequence: the first slice was from a control rat, followed by a slice from an rat with hyperthermia-induced seizures, which was followed by a slice from control, and so on. Whole-cell recordings of evoked and miniature inhibitory postsynaptic currents were obtained from CA1 pyramidal cell layer in brain slices perfused with ACSF containing either of the following solutions: For evoked IPSCs, 10 mM 2-amino-5-phosphovaleric acid (APV) and 5 mM 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX)(both from Tocris, Ballwin, Missouri); for mIPSCs, 10 mM APV, 5 mM CNQX and 1 mM tetrodotoxin (TTX; Calbiochem. La Jolla, California). To evoke IPSCs and field responses, constant-current stimuli (20 μsec) were applied at 0.1 Hz through a bipolar 90-μm (tip separation) tungsten stimulating electrode placed in the stratum radiatum of CA1. To evoke and compare IPSCs in brain slices from control and hyperthermic rats in a reproducible and meaningful manner, the placement and the distance of the recording electrode and the stimulating electrode were kept constant: The stimulating electrode was placed in the stratum radiatum in the CA1 region proximal to the CA1/CA3 border, and cells were recorded at approximately 100 μm away from the stimulating electrode. This experimental approach standardized the recording and stimulating conditions, as also shown by the almost-identical responses obtained from normothermic and hyperthermic controls (Fig. 2) and by the similarity of the control and HT responses recorded from different rats during separate experiments (Figs. 2 and 4). To ensure that only monosynaptic responses were considered, data were invariably rejected if the latency was greater than 3.1 ms or if an inflexion point could be observed on the rising phase of the evoked IPSC. Field recordings of orthodromic population spikes in the pyramidal cell layer of the CA1 area were conducted using patch pipettes filled with ACSF.

Patch pipettes were pulled from borosilicate (KG-33) glass capillary tubing (1.5-mm outer diameter; Garner Glass, Claremont, California) with a Narishige PP-83 two-stage electrode puller. Pipette solutions consisted of 140 mM CsCl, 2 mM $MgCl_2$ and 10 mM HEPES and, in some experiments, 3 mM QX-314.

Analysis

Recordings were filtered at 3 kHz before digitization at 20 kHz by a personal computer for analysis using Strathclyde Electrophysiology Software (courtesy of J. Dempster, University of Strathclyde) and Synapse software (courtesy of Y. De Koninck, McGill University). Individual mIPSCs were detected with a software trigger that has been described[49]. All of the detected events were analyzed, and any ‘noise’ that spuriously met trigger specifications was rejected. Cumulative probability plots of mIPSCs were constructed by pooling 100 mIPSCs from each cell (Fig. 3). Statistical analyses (*t*-test; for cumulative probability distributions, Kolmogorov-Smirnov test) used Sigma plot or SPSS for Windows, with $P < 0.05$ considered statistically significant. Data are presented as mean ± s.e.m.

Hyperthermia-induced seizures

The hyperthermia-induced seizure paradigm has been described in detail[11]. On postnatal day 10, the core temperature of rat pups ($n = 269$) was raised using a regulated stream of moderately heated air. Pups were placed on the floor of a 3-liter glass container, and the air stream was directed about 50 cm above them. Rectal temperatures were measured at baseline, at 2-min intervals, and at the onset of hyperthermic seizures, which occur in almost

all rats. Hyperthermia (defined as core temperature greater than 39.5 °C) was maintained for 30 min, with the goal of a core temperature of 41–42 °C, and the presence and duration of seizures for each pup were noted at 2-min intervals. Seizure duration averaged 22.8 ± 0.3 min, and threshold temperature to seizure onset averaged 41.1 °C. After the hyperthermia period, rats were placed on a cool surface, monitored for 15 min, and then returned to home cages. Pups that were sedated because of pentobarbital pretreatment were hydrated orally and returned to their cages when their behavior returned to normal (typically less than 1 h). The behavioral seizures in this paradigm are stereotyped and easily monitored, and have been shown to correlate with EEG rhythmic epileptiform discharges from the hippocampus and amygdala[11]. Kainic acid-induced seizures were elicited by the intra-peritoneal injection of 1.2 mg/kg kainic acid in postnatal day 10 rats as described[50].

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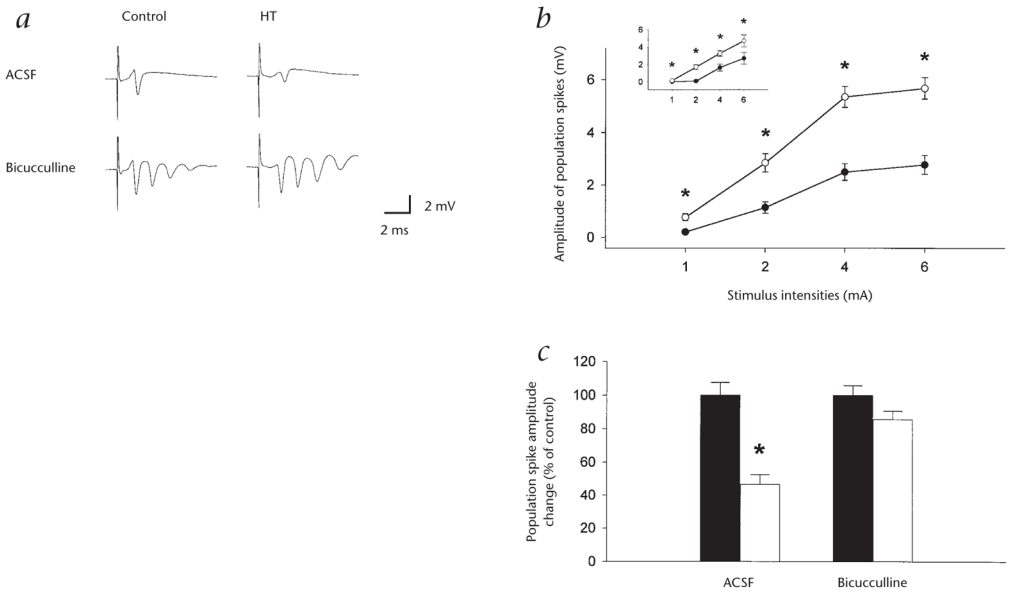

Fig. 1.

Hyperthermia-induced seizures result in a $GABA_A$ receptor-dependent, long-term depression of the discharges of CA1 pyramidal cells. ***a***, Upper row, orthodromic responses recorded in the CA1 pyramidal cell layer of control rats and of rats with hyperthermia-induced seizures (HT), 1 week after HT, in control ACSF. Lower row, responses from the same brain slices in the presence of the $GABA_A$ receptor blocker bicuculline. The population spike is considerably smaller in the HT rat than in the control rat in bicuculline-free, but not in bicuculline-containing ACSF. Thus, the difference between the population spikes in the upper row was mostly abolished by bicuculline. ***b***, The depression of the population spikes in CA1 was significant (*, $P < 0.05$) at all stimulation intensities in control ACSF. Control rats (○), $n = 14$ brain slices; HT rats (●), $n = 16$ brain slices. Inset, The depression of the population spikes was also seen in the granule cell layer of the dentate gyrus after stimulation of the preporant path. Control rats, $n = 7$ brain slices; HT rats, $n = 8$ brain slices. ***c***, The population spike amplitude in the CA1 pyramidal cell layer from the HT rats in control ACSF is significantly smaller than that of control rats (*, $P < 0.05$), whereas there is no difference in the population spike amplitudes of HT and the control rats in the presence of the bicuculline (stimulus intensity, 4 mA). Data are expressed as a percent of population spike amplitudes measured in control brain slices (■, control; □, HT). These data indicate that there is a long-lasting depression of population responses of CA1 pyramidal cells of the experimental rats, and that this depression is mainly due to the enhanced $GABA_A$ receptor-mediated 'feed-forward' inhibitory input to CA1 pyramidal cells.

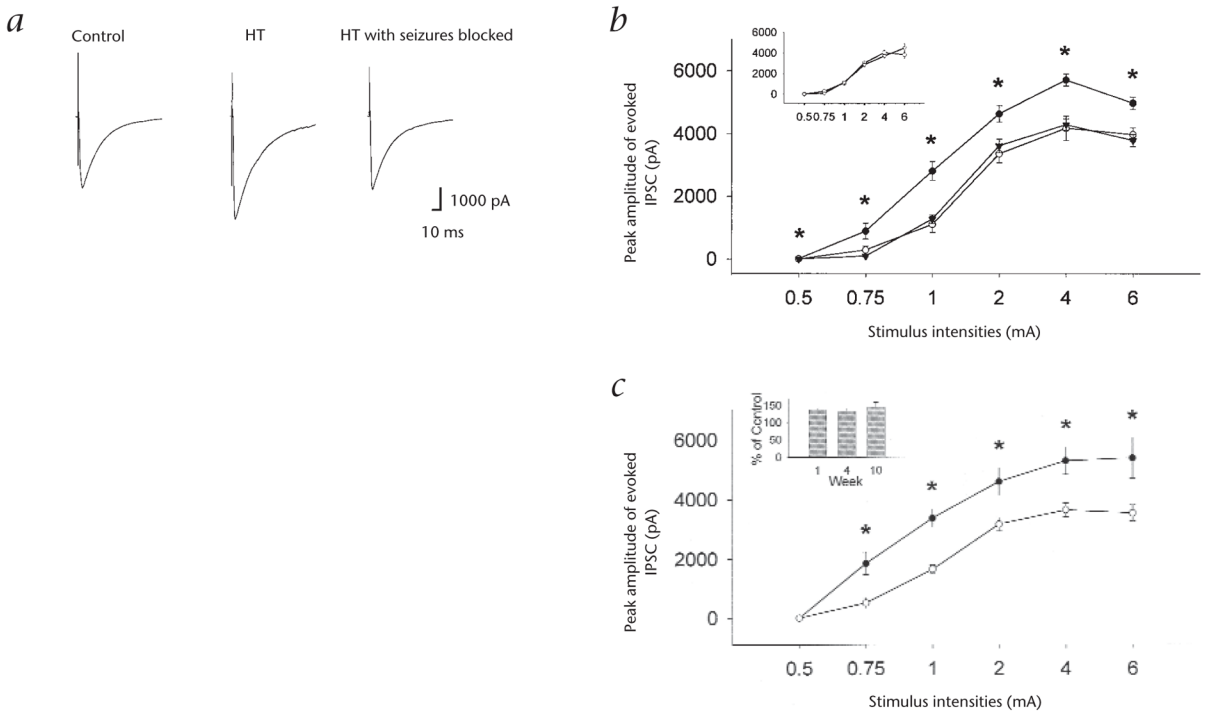

Fig. 2.

The $GABA_A$ receptor-mediated inhibitory postsynaptic currents in CA1 pyramidal cells is enhanced in rats that experienced seizures. ***a***, Evoked IPSCs 1 week after hyperthermia-induced seizures from an age-matched control rat (Control), from a rat that experienced hyperthermic seizures (HT) and from a hyperthermic control rat exposed to hyperthermia but whose seizures were blocked with a barbiturate (HT with seizures blocked). ***b***, Summary plot of data obtained from recordings similar to those in *a*. The evoked IPSCs of brain slices from HT rats were invariably larger in amplitude than those of either normothermic or hyperthermic controls, at all stimulation intensities (including the smallest stimulation intensity, at which the responses seem to overlap because of the relatively large scale on the vertical axis). Control, (○) $n = 6$ cells; HT, (●) $n = 6$ cells; hyperthermic control, (▼) $n = 9$ cells. *, $P < 0.05$. These results were replicated in brain slices from another set of control and HT rats used to test the effectiveness of various protein kinase blockers (Fig. 4). Because the hyperthermic controls[11,12] were identical to normothermic controls, it is the hyperthermic seizures, and not the hyperthermia itself, that result in long-lasting potentiation of the evoked IPSCs. Inset, pentobarbital injection (at the same dose as used for the hyperthermic controls) did not have any effect on the amplitude of the evoked IPSCs. Control, (○) $n = 6$ cells; controls with pentobarbital, (▽) $n = 6$ cells. ***c***, The potentiation of the IPSCs was also found even 10 weeks after hyperthermia-induced seizures, indicating that the alterations in inhibitory neurotransmission are long-lasting; $n = 6$ cells for both HT (●) and control (○). *, $P < 0.05$. Inset, The degree of potentiation (relative to age-matched controls) was essentially unchanged at various times after hyperthermia-induced seizures; stimulation intensity, 2 mA.

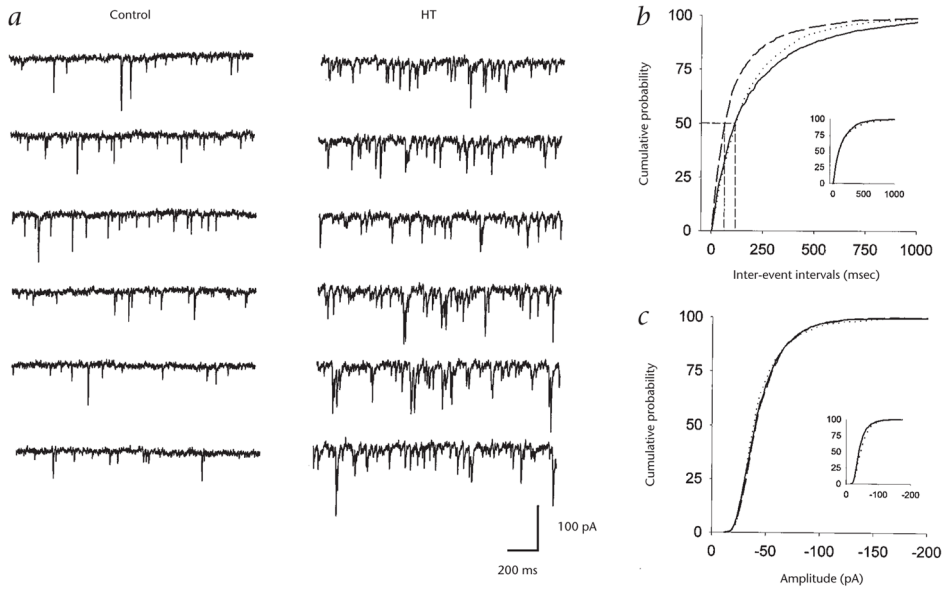

Fig. 3.

The frequency, but not the amplitude, of the miniature IPSCs (mIPSCs) is increased in rats that experienced seizures. ***a***, mIPSCs from CA1 pyramidal cells from a control rat and from a rat that experienced hyperthermia-induced seizures 1 week before recording (HT). The frequency of the miniature IPSCs in CA1 pyramidal cells was considerably increased in HT rats. ***b*** and ***c***, Summary data of the mIPSC inter-event interval (*b*) and amplitude (*c*) from cells similar to those in *a* as well as from cells from hyperthermic control rats ($n = 14$ cells in all three groups). The near-doubling of the frequency of the mIPSCs in the HT group, corresponding to a large decrease in the inter-event interval in *b*: median inter-event interval in normothermic control, 113.1 ms (solid lines); in HT, 64.6 ms (dashed lines); in hyperthermic control, 119.9 ms (dotted lines), without any change in the amplitude (or kinetics), indicates that the potentiation of the IPSCs has a presynaptic locus. Insets, 1 week after kainic acid-induced seizures at postnatal day 10, no equivalent alterations in the frequency of the mIPSCs could be seen (in addition, in contrast to the unchanged mIPSC amplitude after hyperthermia-induced seizures, there was a small, but statistically significant increase in the amplitude of the mIPSCs after kainate injection, compared with that of littermate controls); $n = 12$ cells in both the control (solid lines) and kainate-injected (dotted lines) groups.

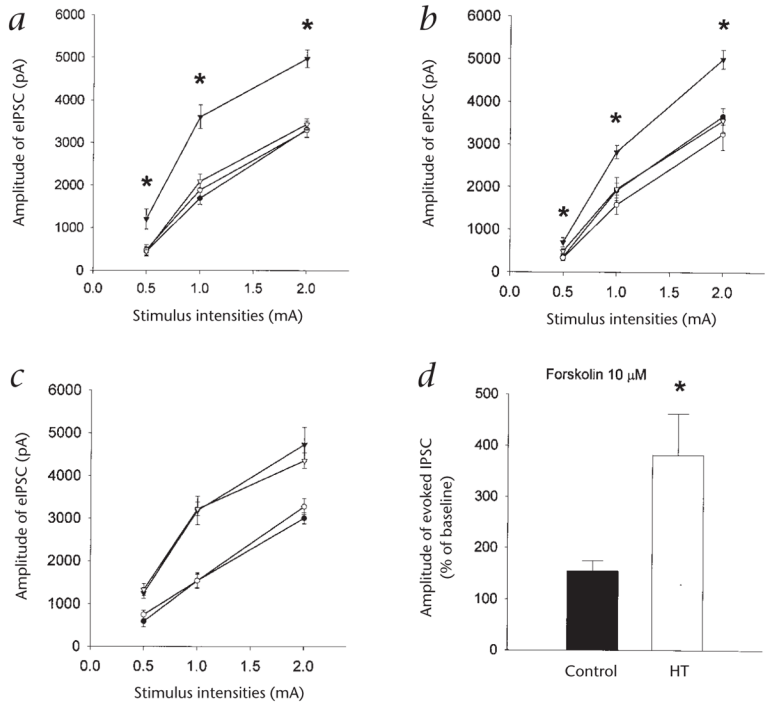

Fig. 4.

The effects of protein kinase blockers on the enhanced amplitude of the evoked IPSCs in CA1 pyramidal cells after hyperthermia-induced seizures. ***a***, Staurosporin (0.5 μM; 2 h of incubation) abolished the difference between the evoked IPSC amplitudes in CA1 cells from control and experimental (HT) rats (1 week after seizures). The amplitude of the evoked IPSCs without staurosporin from the same HT rats was increased (as expected from Fig. 2). Control with staurosporin (○), $n = 7$ cells; control without staurosporin (●), $n = 6$ cells; HT with staurosporin (△), $n = 8$ cells; HT without staurosporin (▲), $n = 7$ cells. *, $P < 0.05$. The ability of staurosporin to abolish the difference between control and HT rats indicates the involvement of a protein kinase in the presynaptic potentiation of the $GABA_A$ responses in CA1 cells after hyperthermia-induced seizures. ***b***, The PKA-specific inhibitor Rp-cAMPS (100 μM) also abolished the enhanced evoked IPSC amplitude recorded from CA1 pyramidal cells of HT rats 1 week after hyperthermia-induced seizures. Control with Rp-cAMPS (○), $n = 7$ cells; control without Rp-cAMPS (●), $n = 7$ cells; HT with Rp-cAMPS (△), $n = 7$ cells; HT without Rp-cAMPS (▲), $n = 8$ cells; the brain slices were incubated in Rp-cAMPS for 2 hours. *, $P < 0.05$. ***c***, Incubation of the brain slices with the specific PKC inhibitor calphostin C (1 μM, 2 h of incubation) did not have any effect on the increased amplitude of the monosynaptically evoked IPSCs in CA1 pyramidal cells from HT or control rats 1 week after hyperthermia-induced seizures. Control with calphostin-C (○), $n = 6$ cells; control without calphostin-C (●), $n = 6$ cells; HT with calphostin-C (△), $n = 9$ cells; HT without calphostin-C (▲), $n = 6$ cells. In contrast, the same concentration of calphostin was able to block the PDBU-induced increase in the evoked IPSC amplitude, indicating that the PKC antagonist was effectively blocking PKC activity in these experiments. ***d***, Forskolin (10 μM) enhanced the amplitude of the evoked IPSCs in CA1 cells from HT rats to a significantly greater degree than in control rats. Evoked IPSC after forskolin with respect to the amplitude of the evoked IPSC before forskolin: control, 155.0 ± 20%; HT, 381.0 ± 81%; $n = 5$ for both groups. *, $P < 0.05$. Thus PKA, but not PKC, is involved in the presynaptic enhancement of the inhibitory neurotransmission after HT-induced seizures.